09256156 2-25-98 #23

6-23-03

1. (Twice amended) An antibody-based fusion protein comprising at least a portion of a CH2 domain, wherein said portion comprises a domain required for immunoglobulin protection receptor (FcRp) binding affinity, linked to a non-Ig protein, wherein said CH2 domain is an IgG1 or an IgG3 CH2 domain comprising a mutation or a deletion that reduces binding affinity for an Fc receptor, and said antibody-based fusion protein has a longer circulating half-life *in vivo* than [~~an~~] said antibody-based fusion protein without said mutation or deletion.

2. (Amended) An [~~The~~] antibody-based fusion protein comprising at least a portion of a CH2 domain linked to a non-Ig protein [~~of claim 1~~], wherein said [~~portion of heavy chain comprises at least the~~] CH2 domain is [~~of~~] an IgG2 CH2 domain [~~or IgG4 constant region~~], and said antibody-based fusion protein has a longer circulating half-life *in vivo* than an antibody-based fusion protein comprising a portion of an IgG1 CH2 domain linked to said non-Ig protein.

X. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain comprises at least a portion of an IgG1 constant region having a mutation or a deletion at one or more amino acid selected from the group consisting of Leu_{234}, Leu_{235}, Gly_{236}, Gly_{237}, Asn_{297}, and Pro_{331}.

X. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain comprises at least a portion of an IgG3 constant region having a mutation or a deletion at one or more amino acid selected from the group consisting of Leu_{281}, Leu_{282}, Gly_{283}, Gly_{284}, Asn_{344}, and Pro_{378}.

X. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain has substantially reduced binding affinity for a Fc receptor selected from the group consisting of FcγRI, FcγRII and FcγRIII.

X. The antibody-based fusion protein of claim 1, wherein said second non-Ig protein is selected from the group consisting of a cytokine, a ligand-binding protein, and a protein toxin.

X. The antibody-based fusion protein of claim 1, wherein said cytokine is selected from the group consisting of a tumor necrosis factor, an interleukin, and a lymphokine.

X. The antibody-based fusion protein of claim 8, wherein said tumor necrosis factor is tumor necrosis factor alpha.

X. The antibody-based fusion protein of claim 8, wherein said interleukin is interleukin-2.

X. The antibody-based fusion protein of claim 8, wherein said lymphokine is a lymphotoxin or a colony stimulating factor.

X. The antibody-based fusion protein of claim 11, wherein said colony stimulating factor is a granulocyte-macrophage colony stimulating factor.

X. The antibody-based fusion protein of claim 1, wherein said ligand-binding protein is selected from the group consisting of CD4, CTLA-4, TNF receptor, and an interleukin receptor.

27. (Amended) An antibody-based fusion protein comprising a variable domain and a portion of [a] an IgG4 CH2 domain, the C-terminus of which is linked to the N-terminus of a non-Ig protein, [~~wherein said CH2 domain is an IgG4 CH2 domain, and~~] wherein said antibody-based fusion protein has a longer circulating half-life *in vivo* than an antibody-based fusion protein comprising a portion of an IgG1 CH2 domain linked to said non-Ig protein.

-continued

CAAAGCCAGG ACCTGCTGGA CCTCGGGCTC GAGGACCTGA GGATGGAGCA GAGAGTCCCC 300

GATGCTCTTG TCTTCACCAT CCAGACCAGG GGGACTGCGG AGCCCATCAC GGTCACCATT 360

GTGCCTGCCT ACAGAGCCCT G 381

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 275 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGATACTTCC AATGTAAAGG CAGGCTCTCC CCAAAATACT ACTTACCACC CTCTGGCTTC 60

CTCAATCCCC AATCTCTTCC TTTGCTCCTT CACTCCTCAG GGCCTTCTCT TCCCAACTCC 120

CAGCCACCCC CTGAGGTCTA TGTGAGCCTG ATCAAGGCCT GCGGTGGTCC TGGAAATTTC 180

TGCCCATCCT TCAGCGAGCT GCAGAGAAAT TTCGTGAAAC ATCGGCCAAC TAAGCTGAAG 240

AGCCTCCTGC GCCTGGTGAA ACACTGGTAC CAGCA 275

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 243 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTATGTGAAA GCCAGGTCCC CCAGAGCCAA TCTGCCCCCT CTCTATGCTC TTGAACTTCT 60

AACCATCTAT GCYTGGGAAA TGGGTACTGA AGAAGACGAG AATTTCATGT TGGACGAAGG 120

CTTCACCACT GTGATGGACC TGCTCCTGGA GTATGAAGTC ATCTGTATCT ACTGGACCAA 180

GTACTACACA CTCCACAATG CAATCATTGA GGATTGTGTC AGAAAACAGC TCAAAAAAGA 240

GAG 243

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 355 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GATATCACAA TTCTCAGTGG CTGGACGAAA TAATTGCCGA GAAGGTTTTT TNCTGGCTTG 60

AAGGCCTTCA AACCATTATA AGCCTGGGCA CCCTTTTCCT GTGTTACAGG CCCATCATCC 120

TGGATCCGGC CGACCCCACC CTCAACGTGG CAGAAGGGTA CAGATGGGAC ATCGTTGCTB 180

CAGAGGGCCT CCCAGTGCCT GAAACAGGAC TGTTGCTATG ACAACAGGGA GAACCCCATC 240

TCCAGCTGGA ACGTGAAGGT AATGGCTCCT CTCTGGGCTT TCAAGGGCTT GAAGGTCAGA 300

ACGACAGATA AACTACTCAG TATTTACTCA TTCAGTTCTG TGTTGATGGA GAACA 355

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1. (Amended) An antibody-based fusion protein [~~with an enhanced circulating half-life,~~] comprising at least a portion of a CH2 domain required for immunoglobulin protection receptor (FcRp) binding affinity, [~~an immunoglobulin (Ig) heavy chain having substantially reduced binding affinity for an Fc receptor, said portion of heavy chain being~~] linked to a [~~second~~] non-Ig protein, wherein said CH2 domain is an IgG1 or an IgG3 CH2 domain comprising a mutation or a deletion that reduces binding affinity for an Fc receptor, and said antibody-based fusion protein [~~having~~] has a longer circulating half-life *in vivo* than an antibody-based fusion protein without said mutation or deletion [~~unlinked second non-Ig protein~~].

2. (Amended) An [~~The~~] antibody-based fusion protein comprising at least a portion of a CH2 domain linked to a non-Ig protein [~~of claim 1~~], wherein said [~~portion of heavy chain comprises at least the~~] CH2 domain is [~~of~~] an IgG2 CH2 domain [~~or IgG4 constant region~~], and said antibody-based fusion protein has a longer circulating half-life *in vivo* than an antibody-based fusion protein comprising a portion of an IgG1 CH2 domain linked to said non-Ig protein.

X. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain comprises at least a portion of an IgG1 constant region having a mutation or a deletion at one or more amino acid selected from the group consisting of Leu_{234}, Leu_{235}, Gly_{236}, Gly_{237}, Asn_{297}, and Pro_{331}.

X. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain comprises at least a portion of an IgG3 constant region having a mutation or a deletion at one or more amino acid selected from the group consisting of Leu_{281}, Leu_{282}, Gly_{283}, Gly_{284}, Asn_{344}, and Pro_{378}.

X. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain has substantially reduced binding affinity for a Fc receptor selected from the group consisting of FcγRI, FcγRII and FcγRIII.

X. The antibody-based fusion protein of claim 1, wherein said second non-Ig protein is selected from the group consisting of a cytokine, a ligand-binding protein, and a protein toxin.

X. The antibody-based fusion protein of claim 1, wherein said cytokine is selected from the group consisting of a tumor necrosis factor, an interleukin, and a lymphokine.

X. The antibody-based fusion protein of claim 8, wherein said tumor necrosis factor is tumor necrosis factor alpha.

X. The antibody-based fusion protein of claim 8, wherein said interleukin is interleukin-2.

X. The antibody-based fusion protein of claim 8, wherein said lymphokine is a lymphotoxin or a colony stimulating factor.

X. The antibody-based fusion protein of claim 11, wherein said colony stimulating factor is a granulocyte-macrophage colony stimulating factor.

X. The antibody-based fusion protein of claim 1, wherein said ligand-binding protein is selected from the group consisting of CD4, CTLA-4, TNF receptor, and an interleukin receptor.

27. (New) An antibody-based fusion protein comprising a variable domain and a portion of a CH2 domain linked to the N-terminus of a non-Ig protein, wherein said CH2 domain is an IgG4 CH2 domain, and said antibody-based fusion protein has a longer circulating half-life *in vivo* than an antibody-based fusion protein comprising a portion of an IgG1 CH2

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1. An antibody-based fusion protein with an enhanced circulating half-life, comprising at least a portion of an immunoglobulin (Ig) heavy chain having substantially reduced binding affinity for an Fc receptor, said portion of heavy chain being linked to a second non-Ig protein, said antibody-based fusion protein having a longer circulating half-life *in vivo* than an unlinked second non-Ig protein.

2. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain comprises at least the CH2 domain of an IgG2 or IgG4 constant region.

3. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain comprises at least a portion of an IgG1 constant region having a mutation or a deletion at one or more amino acid selected from the group consisting of Leu_{234}, Leu_{235}, Gly_{236}, Gly_{237}, Asn_{297}, and Pro_{331}.

4. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain comprises at least a portion of an IgG3 constant region having a mutation or a deletion at one or more amino acid selected from the group consisting of Leu_{281}, Leu_{282}, Gly_{283}, Gly_{284}, Asn_{344}, and Pro_{378}.

5. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain further has binding affinity for an immunoglobulin protection receptor.

6. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain has substantially reduced binding affinity for a Fc receptor selected from the group consisting of FcγRI, FcγRII and FcγRIII.

7. The antibody-based fusion protein of claim 1, wherein said second non-Ig protein is selected from the group consisting of a cytokine, a ligand-binding protein, and a protein toxin.

8. The antibody-based fusion protein of claim 1, wherein said cytokine is selected from the group consisting of a tumor necrosis factor, an interleukin, and a lymphokine.

9. The antibody-based fusion protein of claim 8, wherein said tumor necrosis factor is tumor necrosis factor alpha.

10. The antibody-based fusion protein of claim 8, wherein said interleukin is interleukin-2.

11. The antibody-based fusion protein of claim 8, wherein said lymphokine is a lymphotoxin or a colony stimulating factor.

12. The antibody-based fusion protein of claim 11, wherein said colony stimulating factor is a granulocyte-macrophage colony stimulating factor.

13. The antibody-based fusion protein of claim 1, wherein said ligand-binding protein is selected from the group consisting of CD4, CTLA-4, TNF receptor, and an interleukin receptor.

25. The antibody-based fusion protein of claim 24, wherein said colony stimulating factor is a granulocyte-macrophage colony stimulating factor.

(HA) An increased half-life.

09/256,156 Pending Claims

lines 13-24 p. 8

1. An antibody-based fusion protein comprising at least a portion of a CH2 domain, wherein said portion comprises a domain required for immunoglobulin protection receptor (FcRp) binding affinity, linked to a non-Ig protein, wherein said CH2 domain is an IgG1 or an IgG3 CH2 domain comprising a mutation or a deletion that reduces binding affinity for an Fc receptor, and said antibody-based fusion protein has a longer circulating half-life in vivo than said antibody-based fusion protein without said mutation or deletion.

2. An antibody-based fusion protein comprising at least a portion of a CH2 domain linked to a non-Ig protein, wherein said CH2 domain is an IgG2 CH2 domain, and said antibody-based fusion protein has a longer circulating half-life in vivo than an antibody-based fusion protein comprising a portion of an IgG1 CH2 domain linked to said non-Ig protein → Junghans + Canfield- 103

Canfield

3. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain comprises at least a portion of an IgG1 constant region having a mutation or a deletion at one or more amino acid selected from the group consisting of [redacted], Leu_{235}, Gly_{236}, Asn_{297}, and [redacted] ⇒ Junghans + Canfield

4. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain comprises at least a portion of an IgG3 constant region having a mutation or a deletion at one or more amino acid selected from the group consisting of Leu_{281}, Leu_{282}, Gly_{283}, Gly_{284}, Asn_{344}, and Pro_{378}.

5. The antibody-based fusion protein of claim 1, wherein said portion of heavy chain has substantially reduced binding affinity for a Fc receptor selected from the group consisting of FcγRI, FcγRII and FcγRIII.

103 6. The antibody-based fusion protein of claim 1, wherein said second non-Ig protein is selected from the group consisting of a cytokine, a ligand-binding protein, and a protein toxin.

103 7. The antibody-based fusion protein of claim 1, wherein said cytokine is selected from the group consisting of a tumor necrosis factor, an interleukin, and a lymphokine.

103 8. The antibody-based fusion protein of claim 8, wherein said tumor necrosis factor is tumor necrosis factor alpha.

103 9. The antibody-based fusion protein of claim 8, wherein said interleukin is interleukin-2.

11. The antibody-based fusion protein of claim 8, wherein said lymphokine is a lymphotoxin or a colony stimulating factor.

103 12. The antibody-based fusion protein of claim 11, wherein said colony stimulating factor is a granulocyte-macrophage colony stimulating factor.

103 13. The antibody-based fusion protein of claim 1, wherein said ligand-binding protein is selected from the group consisting of CD4, CTLA-4, TNF receptor, and an interleukin receptor.

27. An antibody-based fusion protein comprising a variable domain and a portion of an IgG4 CH2 domain, the C-terminus of which is linked to the N-terminus of a non-Ig protein, wherein said antibody-based fusion protein has a longer circulating half-life in vivo than an antibody-based fusion protein comprising a portion of an IgG1 CH2 domain linked to said non-Ig protein.

103 28. The antibody-based fusion protein of claim 13, wherein said interleukin receptor is selected from the group consisting of interleukin-1 and interleukin-4 receptors.

103 = Junghans - new
Gillies - old

~~~CH2 domain~~~ ⇒ IgG1 or IgG3

~~~FcRp binding affinity~~~

Key seems to be reduced or no affinity to Fc receptors

Non-Ig — [CH2 domain of IgG1 or 3 w/mutation to ↓ binding to FcR AND FcRp binding domain]

Non-Ig — [IgG2 CH2 domain w/longer ½ life than IgG1]